

## Comparison between The chemical components of kidney stone between Males and Females

Sahib Ali Mahdi AL-Atrakchi, Karbala university, College of Medicine

Salih Mahdi Haddawi, Karbala university ,College of science, Department of Chemistry

Haider Naji Kudhair Kufa University, College of science, Department of Chemistry

**Key word:** Chemical component of kidney stone

(Received November2011 , Accepted June2012 )

### Abstract:

The present investigation was designed to qualitative analysis of renal calculi. Twenty-eight calculi were obtained from (8) females and (20) males afflicted with nephrolithiasis. Their ages ranged from (7-60) years. Also Qualitative assay of renal stone appeared higher percentages uric acid , calcium, oxalate, phosphours and ammonium in calculi of males when compared with that of females.

### المقارنة بين المكونات الكيميائية للحصاة الكلى بين الرجال والنساء

صاحب علي مهدي الاطرقجي

جامعة كربلاء كلية الطب

صالح مهدي حداوي جامعة كربلاء كلية العلوم

حيدر ناجي خضير جامعة الكوفة كلية العلوم

**مفتاح البحث:** المكونات الكيميائية لحصاة الكلى

**الخلاصة:**

تم في هذه الدراسة إجراء التحليل النوعي لثمان وعشرون حصاة مستخرجة من الكلى وكانت لعشرون رجل وثمان امرأة مصابين بالحصى الكلوي وبأعمار تتراوح من (7-60) سنة. حيث أظهرت دراسة التحليل النوعي للحصاة المدروسة زيادة نسبة كل من حامض اليورك, الكالسيوم, الاوكزالات, الفسفور والامونيوم لحصاة الذكور عند مقارنتها مع حصاة الإناث.

### Introduction:

Kidney stone are made of salts and minerals that stick together to form small pebbles. Kidney stones typically leave the body by passage in the urine stream, and many stone are formed and passed without causing symptoms.<sup>(1)</sup>

If stones grow to sufficient size before passage on the order of at least (2-3) millimeters they can cause obstruction of the ureter. The resulting obstruction causes dilation or stretching of the upper ureter as well as muscle spasm of the ureter , trying to move the stone <sup>(2)</sup> This leads to pain, most

commonly felt in the flank , lower abdomen and groin. Renal colic can be associated with nausea, Vomiting and blood in the urine . There are several types of Kidney stones based on the type of crystals of which they included:

- 1- calcium oxalate stone.
- 2-uric acid stone.
- 3- struvite stone.
- 4- other stones.<sup>(3)</sup>

Kidney stones can be due to underlying metabolic conditions , such as renal tubular acidosis,<sup>(4)</sup> Dent's disease,<sup>(5)</sup> hyperparathyroidism and Medullary sponge kidney stone,<sup>(6)</sup> increase global temperatures will lead to greater future prevalence of kidney stone .<sup>(1)</sup>

The aims of this study is to evaluate the composition of kidney stone qualitatively and to predict the relation between kidney stone and change to some biochemical parameters.

## **Material and Methods:**

### **Patients and control subjects:**

During the period from July to December (2009) .Twenty- eight stones obtained from patients afflicted with urolithiasis. They were 20 males and 8 females .Their ages ranged from (7\_60) years. All of them were attended to the Al-Sadder Teaching Hospital in Najaf city. Stones were obtained by surgery .

The stones were washed to remove any dried blood or other matter, Dried in an incubator at 37 °C and weighted . They were pulverized in a motor ,A sample of the powdered stone was weight(0.1gm). The residue after drying was divided into aliquots for chemical analysis.The stone were subjected to quantitative analysis to determine composition.

### **. Qualitative Analysis of stone:**

For a complete chemical analysis, small portion (0.1gm) of powdered calculi were added into 5 test tubes to check for the individuals constituents according to the following approaches:

**Reagent:** 1- 10% HCL: prepared by dilution 10ml of concentrated HCL to 100ml with distilled water 2- 20% NaOH : prepared by dissolving 20gm of NaOH in 100 ml of distilled water.

### **Calcium:**

- 1-five drops of hydrochloride acid (10%) were added to the first test tube.
- 2-Two drops of sodium hydroxide(20%) were added to the same test tube . the appearance of white cloudy precipitate was interpreted as positive for the presence of calcium.<sup>(7)</sup>

### **Oxalate and Carbonate:**

- 1-To a second test tube few drops of hydrochloride acid (10%) was added.
- 2-To the residue that remained after heating and cooling , a few drops of hydrochloride acid (10%) were added. The effervescence at this point when there was none before heating were showed the concentrated of oxalate in stone.<sup>(8)</sup> The appearance of tiny bubbles was interpreted a positive for carbonate.

The appearance of tiny bubbles was interpreted a positive for carbonate.

### **Reagent :**

1-2.5 M NaOH : was prepared by dilution of 156.3ml of 4 N NaOH to 250 ml of distilled water. This solution was used after previous standardization with Hydrochloride.

### **Uric acid :**

- 1-To a small amount of the pulverized stone ,1 to 2 drops of concentrated nitric acid was added.

2-The solution was evaporated slowly just to dryness, a pink-orange color was formed in the presence of uric acid .

3-The tube was cooled and several drops of concentrated ammonium hydroxide were poured, a purple color was developed that indicates the presence of uric acid. <sup>(9)</sup>

#### **Reagent:**

1-Ammonium molybdate (3.5%): 3.5 g of ammonium molybdate was dissolved in a solution prepared by mixing (25) ml of concentrated nitric acid with (75) ml.

#### **Phosphate:**

1-To the fifth test tube 3 drops of 3.5 % ammonium molybdate in 25% nitric acid was added. 2-The tube was heated, the appearance of distinct yellow color was interpreted as positive for phosphate. <sup>(10)</sup>

#### **Reagents:**

1-0.6M HCL : prepared by dilution of 4.98 ml of concentrated HCL to 100ml of distilled water . 2-2.5M NaOH. This solution was used after previous standardization with Hydrochloride

#### **Ammonium ion :**

A ammonium ion was detected by the following method :

1-A small amount of the powdered stone was heated with 2ml of 0.6 M hydrochloric acid.

2-the tube was cooled and neutralized with 2.5M sodium hydroxide .

3-Then 0.5 ML of Nessler solution was added. Nessler solution was prepared by dissolving 100g of mercuric iodide (HgI<sub>2</sub>) and 70g of potassium iodide (KI) in 400ml of distilled water . The mixture was rotated until a complete dissolving ; 100g of NaOH was dissolved in about 500ml of distilled water. The latter was cooled thoroughly and added with constant shaking to the first mixture. The solution was made up to one liter with distilled water. dissolving 100g of mercuric iodide (HgI<sub>2</sub>) and 70g of potassium iodide (KI) in 400ml of distilled water . The mixture was rotated until a complete dissolving ; 100g of NaOH was dissolved in about 500ml of distilled water. The latter was cooled thoroughly and added with constant shaking to the first mixture. The solution was made up to one liter with distilled water.

4-The appearance of orange –brown precipitate indicated the presence of ammonium in the sample. <sup>(9)</sup>

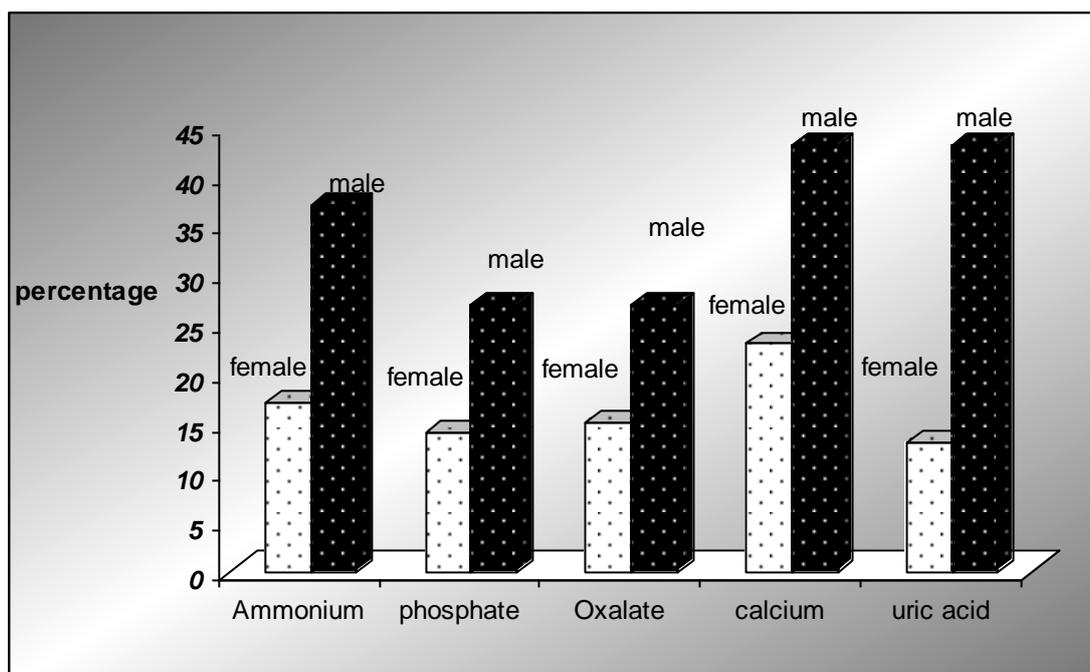
#### **Results:**

##### **The description of mean percentage of kidney stone compositions:**

In the current study , 24 urinary calculi were obtained from patients afflicted with Urolithiasis. These calculi were examined for their shapes. Some staghorn stone, mulberry stone, jack stone which were examined. Composition and constituents were measured by quality analyzed of urinary calculi.

##### **distribution of individual components in kidney stone:**

The frequency of individual component in the quality analyzed kidney stone was expressed in histograms Fig(3.1). The result of calcium and oxalate a higher values in compared with those of females. Uric acid percentages were found to be elevated in males compared with females patients. Phosphate and ammonium showed a higher percentage of these constituent in children compared with patients.



**Fig (3.1) the frequency of individual component in the quality analysis kidney stone.**

### Discussion:

#### The description of mean percentage of kidney stone Composition:

Kidney stone formation is a complex process, Including crystal Nucleation growth, and aggregation and crystal retention within the renal tubules. (11,12) Crystals form in the urine that supersaturated with particular salts such as calcium oxalate ,calcium phosphate, and urat . (13) calcium oxalate is the most common crystalline components of calculi. calculi formation is crystallization of calcium oxalate supersaturated is directly related to the of magnesium and citrate. (14)

The result of the current study indicated a higher average of calcium and oxalate in male when compared with female patients. However these findings agreement with Hodgkin son. (15) had stated a higher average of calcium oxalate from male compared with female patients. The reason were illustrated in the followed paragraph which related to the fasters in urolithiasis, Such as infection and occurrence an alteration in the excretion of urokinase and sialidase activity.

29%of all calculi contain calcium phosphate mostly in the form of hydroxy apatite. (16) all calcium based calculi, serving as site for heterogeneous nucleation. (17) The other mechanism by which bacterial infection can induce calculi formation is by an alteration in the excretion of the enzymes. Du Toil et al.(18) had suggested that a factor in Urolithiasis is an alteration in the excretion of the enzymes urokinase and sialidase. decreased urokinase and increased sialidase in urine leads to the formation of mineralizable matrix. Bacterial infection with proteous mirabilis and E.coli decrease urokinase and increase sialidase activity.

Up to one third of patients with calcium calculi have a history of urinary tract infection, usually associated with E.coli . (19)

The deficiency of intestinal bacteria naturally digest oxalate may be the cause of the elevated prevalence of calcium oxalate calculi in females.

When the bacteria are lost due to antibiotic use, the patients is likely to have increased oxalate absorption. (20) Other factors may to the sex and the seasonal variation . (21) In men, the prostate gland produces secretions that slow bacterial growth.

Women are found to have a greater occurrence of urinary tract infections Primarily. use the urethra is short ,making it easier for bacteria to reach the bladder (22,23) increased phosphate, and ammonium average in children patient. However it can be concluded that the abnormalities in children such as vesicoureteral reflex, urinary obstruction, and present hematuria that made them with high probability to form infections calculi. (24)

The current investigation demonstrated a high average of uric acid in male than the female patients. The variation was seemed to be age dependent phenomenon. Several reports demonstrated increased protein average in females compared with males. (25) Some authors pointed out that elevated protein content in calculi of female patients may belong to the E.coli infection., (26) In some reports, It has been suggested that cystine stone occur only in the patients with cystinuria. (27) In addition, It has been found that cystinuria accounting for 6% to 8% of children urinary calculi causes . (28, 29)

#### **Distribution of individual component in kidney stone:**

calcium and oxalate percentages were found to be higher than that of phosphate in the examined calculi. It is believed that the predominant calcium and oxalate percentages in calculi are related to the nutritional factor in population High protien in take of animal origin contributes to hyperuricosuria,Hypercalciuria , and hypocitraturia. (30,31)

In the past it is recommended calcium restriction to avoid hypercalciuria . (32) However, it has been found a reduction in urinary oxalate level associated with increased in take of dietary calcium. (33) Elevated dietary oxalate may cause raised oxalate excretion and induces calcium calculi formation . (34, 35)

The restriction of oxalate intake has been shown to reduce the urinary oxalate excretion ,but do not prevent calculi formation . (36,37)

Uric acid percentages in calculi were found to have high values with respect to other components. This finding may belong to the over ingestion of purine-rich foods. (38) Dehydration may be involved as essential cause for the super saturation of urine with respect to uric acid . (39)

The pH of urine may be implicated as a directing factor for the super -saturation of urine and crystals formation. (40,41)

## **References:**

- (1)- Parmar, M.S , Kidney stones. *BMJ*, 328(7453): 1420–1424. (2004).
- (2)-Collins, C and Edward, A Short Course in Medical Terminology. Lippincott Williams and Wilkins. 358. (2005).
- (3)- John R , Asplin :Harrison's Principles of Internal Medicine .Electronic version 15th Edition. 283-402. ( 2001).
- (4) -Lloyd, S. E.; Pearce, S. H. S.; Fisher, S. E.; Steinmeyer, K.; Schwappach, Band Scheinman, S. J. A common molecular basis for three inherited kidney stone diseases. *Nature* **379**: 445–449. (1996).
- (5)- Hyperparathyroidism\_ National Endocrine and Metabolic Diseases Information Service. (2006) [www.en.wikipedia.org/wiki/Hyperthyrodism](http://www.en.wikipedia.org/wiki/Hyperthyrodism)
- (6)- Ginalski, J. M.; Portmann, L.and Jaeger, P. Does medullary sponge kidney cause nephrolithiasis?". *American Journal of Roentgenology* **156** (4): p. 872–3. (1991).
- (7)- Beeler M.F,Veith DA, and Morriss R.H. Technical section: Analysis of urinary calculus.*AM.J.clin. path.*34(4):553-559. (1964).
- (8)-Freeman J .A, and Beeler M.F. Kidney stone analysis. *Post grad. Med.*46:51-56. (1969).
- (9)-Fiereck E.A. Analysis of calculi In: Tietz:Fundamentals of clinical chemistry . Tietz(ed)W.B. saunders company. 1023. (1982).
- (10)-Hodgkison ,A. Combined quantitative and quantitative procedure for chemical analysis of urinary calculi. *J clin. Pah.*24:147-151. (1971).
- (11) –Coe, F. L, and parks J.H. pathophysiology of kidney stones and strategies for treatment . *Hosp Pract* 23: 154-168 (1988).
- (12) -kok DJ, and Khan SR. calcium oxalate nephrolithiasis, afre practcle disease of kidney int 46:846-854.(1994).
- (13) -Abomellah M.S ,Abdullah A.A,and Arnold J. Urolithiasis in saudai Arabia.35:31-34. (1996).
- (14) -Hess B, and Tiselius HG. Physical-chemical process in kidney stone . In: Coe Fl, and favus Mj.Eds.disorders of bone and mineral metabolism. 2<sup>nd</sup> ed.Lippincot. Wlkins.Philadelphia. 619-628. (2002).
- (15)-Hodgkinson A, and Williams A. An improved colorimetric procedure for urinary oxalate.*Clin. Chim. Acta.*36:127-132 (1972).
- (16) -Mandel N.S ,and Mandel GS. Urinary tract stone disease in United states veteran population.II.Geographic analysis of variations in composition *Urol.* 1516-1521. (1989).
- (17) -Pak CYC, Eanes ED, and Ruskin B. Spontaneous precipitation of brushite in urine: Evidence that brushite is the nidus of renal stones originating as calcium phosphate. *Proc nat Acad sci USA.*68:1456-1460.(1971).
- (18) -Dug Toil PJ, Van Aswegen C.H, Steyn P.I., Effect of bacteria involved with the pathogenesis of infection induced urolithasis on the urokinase and sialidase (neuraminidase) activity.*Urol Res.*20:393-397 (1992).
- (19)-Holmgren K, Danielson BG, Fellstrm B, et al. The relation between urinary tract infection and stone composition in renal stone formars.*Scand J Urol- Nephrol.*23.p131.(1989).
- (20) Hoppe B,von Unruh G and Laube N. Oxalate degrading bacteria New treatment option for patients with primary and secondary hyperoxaluria.*Urol Res.*33(5):372-338.(2005).

- (21) -parks JH, Barsky R, and Coe FL. Gender differences in seasonal variation of urine stone risk factors. *JUrol*.170:384-338.(2003).
- (22) -Johanson. Clinical notes and examples: Renal physiology of Johnsons text book of medical. Part I V'. Physiology. 2<sup>2</sup>d ed.Lippincott-Raven . Philadelphia . 307-419(1998)
- (23) -[http:// www.prostatitis. Org/](http://www.prostatitis.Org/).(2006).
- (24) -Howes D.S, and Bogner M.P.Urinary tract infections.In:Tintinalli, medicine. A comprehensive study guide. 6 th ed.new York. McGraw-Hill . 606-612.(2004).
- (25) -Boyce WH, and King JS. Present concepts concerning the origin of matrix and stones.*Ann.N.Y.A cad . Sci.*, 104. 563.(1963).
- (26) -Grenabo Hedelin H, and Peterson L, S. Adherence of ureas induced crystals to rabbit epithelium.*Urol res*.16:49-52.(1988)
- (27) -Martin MC,Meyers AAAN and Whalley NA.,Cystine apromoter of growth and aggregation of calcium oxalate crystals in normal undiluted human urine *Urol*.167(1):317-321.(2002).
- (28) -Mitcheil JP. Lithiasis in children. *Eur.Urol*. 7. 121(1981).
- (29) -Rutcheil SD, and Resnick MI.cystine calculi: Diagnosis and management.*Urol. Clin. North. Am*.24(1):163-171.(1997).
- (30) -Breslau NA,Brinkley L and Hill KD .Relationship of animal protien rich diet to kidney stone formation *Clin endocrinol Metab*.66:140-146.(1988).
- (31) -Holmes RP , G oodman HO and Hart LJ.,Relationship of protien intake to urinary oxalate and glycolate excretion. *Kid. Int*44:366-372.(1993).
- (32) -PakCYC .Kidney stone. *Lancet*.351:1797-1801.(1998).
- (33) -Hess B , Jost C and Zipperle L,. High calcium intakes abolishes hyperoxaluria and reduce urinary crystallization during 20-fold normal oxalate load in humas.*Nephrol Dial Transplan*. 13:2241-2247.(1998).
- (34) -Williams HE, and Wandzilak TR. Oxalate synthesis, transport and the hyperoxaluric syndromes. *J Urol* 141.742-747.(1998).
- (35) -Hillman RE, Ege M, and Hillman LS. Increase in oxalate excretion with lipid infusion -evidence for the presence of the glycoxylate shut in humans. *Pediatr RES*.29.296.(1991).
- (36) -Gurhan GC, Willet WC and speizer FE, . Beverage use and risk for kidney stones in women. *Ann. Int .Med*. 128:534-540.(1998).
- (37) - Gurhan GC, Willet WC and Rimm EB .Prospective study of beverage and the risk of kidney stones. *Am. J.Epideminol*.143:240-247.(1996).
- (38) -Rodgers Al, and Lewandowski S. Effect of 5 different diets on urinary risk factors for calcium oxalate kidney stone formation:evidence of different renal handling mechanism in different race group . *J Urol*. 168(3):931-936. (2002).
- (39) -Lash J.P, Cowell G, and Arruda JAL. Calcium nephrolithiasis and renal tubular acidosis. In: Coe F.L, Favus M.J.(eds). Disorder of bone and mineral metabolism.2<sup>2</sup>d ed. Lippincot. Williams and Wilkins. Philadephia. 717-740.(2002).
- (40) -Serio A, and Frails A. Epidemiology of nephrolithiasis. *Nephron*.81 suppl 1.pp26-30.(1999).
- (41) -Moe OW, Abate N, and sakhaee K. Pathophysiology of uric acid nephrolithiasis.*Endocrinal Metab Clin north Am*.31:895-914.(2002).